DATA EVALUATION RECORD

STUDY 2

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CONCLUSIONS

Degradation - Photodegradation in Water

1. This study is scientifically valid and satisfies the Subdivision N data requirement for aqueous photodegradation.

2. Prohexadione calcium degrades through hydrolysis with a half-life ($T_{1/2}$) of 4.4 days in pH5 buffer solution ($T_{1/2}$ = 65 days in pH7 and stable in pH9, MRID 44457782). It degrades through photolysis with a half-life of 23.2 days in pH 9 buffer solution ($T_{1/2}$ = 9.9 days in pH5, data based on this study).

   Radiolabeled prohexadione calcium when continuously exposed to a filtered xenon lamp for 15 days in a pH 9 buffer solution at temperature 25°C degraded with a half-life of 11.6 days (r^2 = 0.84). The half-life is 23.2 days when corrected for an irradiation period of 12 hours irradiation and 12 hours of darkness. Prohexadione calcium was stable in the dark control (see THE REVIEWERS' COMMENT 2) which is in agreement with the hydrolysis study showing that prohexadione calcium is hydrolytically stable in sterile pH 9 aqueous buffer solution (MRID 44457782).

   In a pH 5 buffer solution at 25°C, when continuously exposed to a filtered xenon lamp for 15 days, ^{14}C-prohexadione calcium degraded with a half-life of 3.6 days (r^2 = 0.90). The half-life is 7.2 days when corrected for an irradiation period of 12 hours irradiation and 12 hours of darkness. The corresponding dark control half-life was 5.6 days (see THE REVIEWERS' COMMENT 2). The value is in close agreement with the hydrolysis rate of 4.4 days in pH 5 sterile aqueous buffer solution obtained in hydrolysis study (MRID 44457782). The pH 5 photodegradation half-life corrected for the contribution from dark hydrolysis was 9.9 days.

3. The major photodegradates were tricarballylic acid (at pH 5: up to 48.6% at 169 hours, at pH 9: up to 30.1% at 219 hours posttreatment), citric acid (at pH 5: up to 19.5% at 26 hours posttreatment), and 3-carboxy-5-oxo-hexanoic acid (at pH 9 up to 18.9% at 283 hours posttreatment).

4. The authors proposed the following degradation pathway: after α-cleavage prohexadione calcium oxidizes to despropionyl (BW9054-5376, 2) or another form (7) which undergoes α-cleavage and oxidation to 3-carboxy-5-oxo-hexanoic acid (BX112-M-10, 3, P3) and CH_2(COOH)CH(COOH)CH_2(COOH) (5, P2). 3-carboxy-5-oxo-hexanoic acid photooxidizes to tricarballylic acid (P2, 5) and citric acid (P1, 6).

METHODOLOGY

Cyclohexene ring-labeled [3,5-^{14}C]prohexadione calcium {BAS 125 W; calcium 3-oxido-4-propionyl-5-oxo-3-cyclohexenecarboxylate; radiochemical purity 99.1%; specific activity 15.3 mCi/mmol; p. 9}, dissolved in 0.1 *N* hydrochloric acid:acetonitrile (1.2:8.8, v:v), was added at nominal concentrations of 10-11 ppm to individual samples of filter-sterilized (0.2 µm) pH 5 (citrate) and pH 9 (borate) aqueous buffer solutions (pp. 12, 13; see Comment #2). Sterility was confirmed on agar plates. The individually treated samples were transferred to sterilized, jacketed glass vessels which were capped with Teflon lids fitted with quartz discs, and continuously irradiated for 360 hours using a xenon lamp equipped with a filter to absorb light at wavelengths of <290 nm (p. 11). The incubation temperature was maintained at a target of 25 ± 1 °C by circulating cooled fluid through the jacketed vessels (Figure 6, p. 40). The design of the photolysis vessel did not allow for temperature measurements inside the vessel so a preliminary study was conducted to determine the cooling bath temperature setting required to maintain the incubation temperature at 25 ± 1 °C. To trap volatiles, sterile air was drawn through the system and flushed successively through two 2 *N* NaOH traps, and an ethylene glycol trap (Figure 5, p. 39). Samples were irradiated at a light intensity of 1700 $\mu Em^{-2}S^{-1}$, which was slightly more than the average light intensity on a clear sunny day (measured from April 25 to May 5 between 11 am and 4 pm) at Research Triangle Park, NC (Appendix 1, p. 60). The spectral distribution and light intensity were measured using a spectroradiometer; mean irradiance over the 300- to 1100-nm wavelength range was determined to be 1700 $\mu Em^{-2}s^{-1}$ (p. 11; Appendix 1, p. 61). Comparison graphs of artificial and natural sunlight were provided in the study (Appendix 1, p. 62). The mean measured natural sunlight value at Research Triangle Park, NC, on selected sunny days was 1664 µE (Appendix 1, p. 60). Dark control solutions were placed in screw-cap vials and incubated in darkness at 25 ± 1 °C for 360 hours (p. 13). In a hydrolysis study (MRID 44457782), the parent compound had a half-life of 4.4 days in pH 5 buffer solution and was stable in pH 9 buffer solution. Samples from irradiated pH 5 and 9 buffer systems were removed for analysis at 0, 26, 49, 68, 96, 120, 141, 169, 219, 283 (pH 9), 294 (pH 5), and 362 hours posttreatment (Tables 2, 3; pp. 28, 29); dark control samples were removed at 24, 48, 72, 96, 120, 144, 168, 216, 288, and 360 hours posttreatment (Tables 5, 6; pp. 31, 32).

Triplicate aliquots of each sample solution were analyzed for total radioactivity by LSC at time 0 and at each sampling interval (p. 14). Aliquots of the irradiated and dark control samples were analyzed by HPLC (YMC-PAK ODS-A column) with a mobile phase gradient of acetonitrile plus 0.1% trifluoroacetic acid:water plus 0.1% trifluoroacetic acid (0:100 to 95:5, v:v). Eluent fractions were collected at one-minute intervals and analyzed by LSC (p. 15).

Selected irradiated samples (169, 219, 294 hours) from the pH 5 solution were acidified to pH 3 (H_3PO_4) and concentrated by solid phase extraction (SPE; ENV^+ column; p. 16). The column was eluted with methanol. The eluate was concentrated and aliquots were analyzed by LSC. Aliquots from each sample were combined and analyzed by LSC. An aliquot of the combined sample was methylated with diazomethane, diluted with ethyl acetate and washed with aqueous $NaHCO_3$ (p. 17). The methylated sample was analyzed by LSC and dried (anhydrous Na_2SO_4). An aliquot was concentrated to dryness under nitrogen, redissolved in acetone and analyzed by GC/MS performed in the electron impact mode.

To confirm compound identities, a separate experiment involving the major hydrolysis degradate, despropionyl (BW9054-5376) was conducted (p. 14). Radiolabeled [^{14}C]despropionyl in pH 5 buffer solution was irradiated at 1700 $\mu Em^{-2}S^{-1}$ for up to 68 hours. The irradiated sample was acidified to pH 3 (H_3PO_4) and concentrated by SPE (ENV^+ column; p. 15). The columns were eluted with methanol followed by methanol plus 3% formic acid. All eluates were combined, concentrated by rotary evaporation, redissolved in methanol and methylated with diazomethane (p. 16). The methylated eluate was concentrated to dryness under nitrogen, redissolved in acetone, and analyzed by GC/MS performed in the electron impact mode.

At selected sampling intervals, ethylene glycol trapping solutions were analyzed by LSC (p. 14). To quantify $^{14}CO_2$ in the NaOH trapping solution, aliquots were acidified (H_2SO_4) and evolved radioactivity was trapped and analyzed by LSC.

STUDY AUTHOR'S DATA SUMMARY

pH 5

Cyclohexene ring-labeled [3,5-^{14} C]prohexadione calcium (radiochemical purity 99.1%), at concentrations of 10-11 ppm, degraded with a registrant-calculated half-life of 3.6 days (r^2 = 0.90; p. 96) in pH 5 (citrate) buffer solutions which were continuously irradiated for 362 hours with a xenon lamp and maintained at 25 ± 1 °C. Data for the irradiated samples were reported as percentages of the applied radioactivity, but represent percentages of the time 0 concentration rather than the radioactivity initially applied for each individual sample. In the irradiated samples, the parent compound was initially present at 97.3% of the applied radioactivity, decreased to 57.3% by 26 hours posttreatment and to 42.2% by 49 hours, and to 4.6% at 362 hours posttreatment (Table 2, p. 28). The major degradate, tricarballylic acid (P2; structure presented in Figure 4, p. 38), was initially present in the irradiated samples at 15.0% of the applied radioactivity at 26 hours posttreatment, increased with variability to a maximum of 48.6% by 169 hours posttreatment and was not detected by 219 hours. The major degradate, citric acid (P1; structure presented in Figure 4, p. 38), was initially 1.1% of the applied radioactivity at time 0, increased to a maximum of 19.5% by 26 hours posttreatment, and was 16.2% at 169 hours posttreatment. Tricarballylic acid and citric acid coeluted after 169 hours (see Comment #9); the two degradates were 72.0-72.8% of the applied radioactivity from 219 to 362 hours posttreatment. The minor degradate 3-carboxy-5-oxo-hexanoic acid (P3; BX112-M-10) was initially present in the irradiated samples at 4.3% of the applied radioactivity at 26 hours posttreatment, increased with variability to a maximum of 9.3% of the applied radioactivity by 294 hours posttreatment and was 8.5% at 362 hours posttreatment. The minor degradate 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid (P5; despropionyl; BW9054-5376) was initially present in the irradiated samples at 1.6% of the applied at time 0, increased to a maximum of 4.0% of the applied by 96 hours posttreatment, decreased to 1.7% by 169 hours posttreatment, and was not detected by 219 hours posttreatment. An unidentified degradate (designated as P4) was initially present in the irradiated samples at 0.47% at 26 hours posttreatment, increased to a maximum of 4.6% of the applied radioactivity by 96 hours posttreatment, decreased to 3.5% by

169 hours posttreatment and was not detected by 219 hours posttreatment. Dark control data were reported as a distribution based on the assumption that ≥98.6% of the applied radioactivity (based on the material balance at 360 hours of sample storage) was present at all sampling intervals; time 0 data were not reported for the dark control samples. In the dark control samples, the parent compound was initially present at 81.3% of the applied radioactivity at 24 hours posttreatment, decreased to 46.5% of the applied by 120 hours posttreatment and was 14.5% at 360 hours posttreatment (Table 5, p. 31). The major degradate 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid (H4; despropionyl, BW9054-5376) was initially present in the dark controls at 14.7% of the applied radioactivity at 24 hours posttreatment and increased to a maximum of 81.6% by 360 hours posttreatment. Three unidentified minor degradates (designated as H1, H2 and H3) were ≤ 1.7% of the applied radioactivity.

pH 9

Cyclohexene ring-labeled [3,5-^{14}C]prohexadione calcium (radiochemical purity 99.1%), at concentrations of 10-11 ppm, degraded with a registrant-calculated half-life of 11.4 days (r^2 = 0.85; p. 98) in pH 9 (borate) buffer solutions which were continuously irradiated for 362 hours with a xenon lamp and maintained at 25 ± 1 °C (Table 8, p. 34). In contrast, the parent compound was stable in dark controls (Table 6, p. 32). Data were reported as percentages of the applied radioactivity, but represent percentages of the time 0 concentration rather than the radioactivity initially applied for each individual sample. In the irradiated samples, the parent was initially present at 98.0% of the applied radioactivity. It decreased to 60.2% by 141 hours posttreatment and was 42.9-49.0% from 169 to 362 hours posttreatment (Table 3, p. 29). The major degradate, tricarballylic acid (P2), was initially present in the irradiated samples at 3.2% of the applied radioactivity at 26 hours posttreatment, increased with variability to a maximum of 30.1% by 219 hours posttreatment and was 23.1% at 362 hours posttreatment. The major degradate 3-carboxy-5-oxo-hexanoic acid (P3; BX112-M-10) was initially present in the irradiated samples at 1.0% of the applied radioactivity at 26 hours posttreatment, increased to a maximum of 18.9% by 283 hours posttreatment, and was 18.3% at 362 hours posttreatment. The minor degradate citric acid (P1) was initially present in the irradiated samples at 2.0% of the applied radioactivity at time 0 and was 8.3-8.6% from 120 to 362 hours posttreatment. An unidentified degradate (designated as P4) was detected twice at 1.8% of the applied radioactivity (283 hours) and 1.4% (362 hours). Dark control data were reported as a distribution based on the assumption that ≥103.2% of the applied radioactivity (based on the material balance at 360 hours of sample storage) was present at all sampling intervals; time 0 data were not reported for the dark control samples. In the dark control samples, the parent compound was 95.0-102.2% of the applied radioactivity from 24 to 288 hours posttreatment, and was 85.6% at 360 hours posttreatment (Table 6, p. 32). The minor degradate 3-hydroxy-5-oxo-3-cyclohexene-1-carboxylic acid (H4; despropionyl) was variable and ranged from <LOD to 1.4% from 24 to 360 hours. Three unidentified minor degradates (designated as H1, H2 and H3) were each present at ≤ 6.4% of the applied radioactivity.

The ethylene glycol traps for collecting volatiles did not contain any radioactive residues in either of the tests. Only the NaOH traps contained some ^{14}C-residues which accounted for up to 8.43%

of total applied radioactivity at pH 5 tests and less than 1% of applied radioactivity at pH 9 tests. $^{14}CO_2$ was the main volatile material.

Material balances for the irradiated samples were 91.2-107.8% of the applied radioactivity at pH 5 tests and 93.4-103.5% at pH 9 tests (Table 1, p. 27; see Comment #2). Material balances for the dark control samples were reported for samples collected at 0 and 360 hours only; recoveries were 100.0% and 98.6% at pH 5 tests and 100.0% and 103.2% at pH 9 tests (Table 4, p. 30), respectively.

THE REVIEWERS' COMMENTS

1. Ring-labeled [3,5-^{14} C]prohexadione calcium, at concentrations of 10-11 ppm, degraded with a half-life of 3.6 days ($r^2 = 0.90$) in pH 5 (citrate) buffer solutions and a half-life of 11.4 days ($r^2 = 0.85$) in pH 9 (borate) buffer solutions when continuously irradiated for 362 hours with a xenon lamp and maintained at temperature 25 ± 1 °C. The degradation half-lives corrected for the day:night irradiation period, 12 hours irradiation:12 hours of darkness, are 7.2 days and 23.2 days in pH 5 and pH 9, respectively. The EFED calculated photodegradation half-life corrected for hydrolysis in pH 5 is 9.9 days (the degradation half-life in the irradiated sample corrected for the dark control) and in pH 9 is 23.2 days (prohexadione calcium in the dark control was stable, see Comment 2).

2. The dark control in both pH 5 and 9 were reported as distribution of recovered radioactivity, not as a fraction of applied, based on the assumption that at least 98.6% of applied radioactivity (a value of material balance at 360th day of sample storage measured by TLC) was present at all sampling intervals in pH 5 and 103.2% (a value of material balance at 360 days of sample storage measured by TLC) in pH 9. Additionally, time zero data were missing and the dark control raw data were not included in the report.

   In the dark control at pH 5 prohexadione calcium was present in 81.3% at 24 hours posttreatment, 46.5% at 120 hours, and 14.5% of the applied at 360 hours posttreatment. The EFED calculated dark control half-life was 5.6 days which is in a close agreement with the hydrolysis $T_{1/2}$ of 4.4 days from the hydrolysis study submitted by registrant (MRID 44457782).

   At pH 9 prohexadione calcium was 95.0 to 102.2% of the applied from 24 to 288 hours posttreatment and 85.6% at 360 hours. The last value is a suspected outlier. The study author did not explain why the percentage of radioactivity present as parent at 360 hours was questionably low. Upon exclusion of the last measurement EFED concludes that prohexadione calcium is stable in the dark control at pH 9 which is in agreement with the hydrolysis study (MRID 44457782).

3. Photodegradation study in pH 5 indicates that hydrolysis is the major route of prohexadione degradation (see Comment 2). Prohexadione calcium degrades through hydrolysis with the half-life ($T_{1/2}$) of 4.4 days in pH5 buffer solution ($T_{1/2}$ = 65 days in pH7 and stable in pH9, MRID 44457782) and through photolysis with the half-life of 23.2 days in pH 9 buffer solution ($T_{1/2}$ = 9.9 days in pH5, data based on this study). The aqueous photolysis half-life of 23.2 days, pH 9, should be used to assess photodegradation potential with competing degradation processes.

4. The major aqueous photodegradate, tricarballylic acid, was present at pH 5 at a maximum concentration of 48.6% of the applied radioactivity at 169 hours posttreatment and at pH 9 at 30.1% at 219 hours, citric acid was present at pH 5 at a maximum concentration of at 19.5% at 26 hours posttreatment, and 3-carboxy-5-oxo-hexanoic acid was present at pH 9 at a maximum concentration of at 18.9% of the applied at 283 hours posttreatment.

5. There was a slight variation in the treatment rates among all of the irradiated samples. Individual samples of pH 5 and 9 aqueous buffer solutions were treated separately with parent at concentrations of 10-11 ppm. As a result, concentrations of the parent in each sample cannot be compared over time. A preferred practice is to prepare individual solutions from a single buffer solution treated with the parent compound.

6. A citrate buffer solution was used for the pH 5 buffer solution; it is recommended that acetate or borate buffers be used to minimize buffer effects.

7. The study authors prepared the prohexadione calcium stock solution (dosing solutions) by dissolving 10.6 mg of the compound in a mixture of 0.1N HCl (1.2 ml) and acetonitrile (8.8 ml). The reported aqueous solubilities at 20 °C were 174.0 ppm (distilled water), 1602 ppm (pH 5), and 665 ppm (pH 9; p. 10). Therefore a solubilizing agent is not necessary to increase the chemical water solubility.

8. The study author stated that the GC/MS analysis confirmed that the degradates P1, P2, and P3 were citric acid, tricarballylic acid and 3-carboxy-5-oxo-hexanoic acid, respectively (p. 22). The study author stated that tricarballylic acid (P1) and citric acid (P2) could not be resolved during the analysis of the 219, 294 and 362 hour samples and that the two products co-eluted (p. 20).

9. The authors proposed following degradation pathway: after α-cleavage prohexadione calcium oxidizes to BW9054-5376 (2) or another form (7) which undergoes α-cleavage and oxidation to BX112-M-10 (3, P3) and $CH_2(COOH)CH(COOH)CH_2(COOH)$ (5, P2). BX112-M-10 photooxidizes to tricarallilic acid (P2, 5) and citric acid (P1, 6). The proposed degradation pathway for prohexadione calcium was presented in Figure 24 (p. 58).

10. Method detection limits (MDL) and limits of quantitation (LOQ) were not reported for either LSC or HPLC analyses of the prohexadione calcium and its degradates.

Prohexadione Calcium

DER MRID 444577-83.

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